SOME ASPECTS OF POST-MORTEM CHANGES IN FRESHWATER FISH

DISSERTATION
SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN
ZOOLOGY
OF
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH.

By
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M. Sc. (Alig.)

DEPARTMENT OF ZOOLOGY
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ALIGARH
July, 1975.


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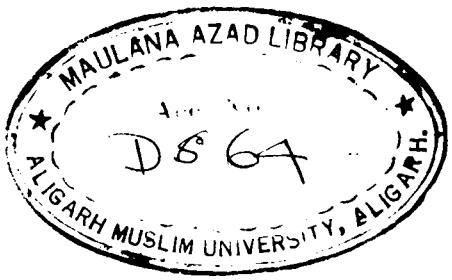

15 DEC 1980

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C O N T E N T S

ACKNOWLEDGMENTS

I owe my gratitude to my supervisor, Dr. A.K. Jafri, for his valuable advice and guidance throughout the course of the present investigation, despite his continued absence from the Department.

I am also much indebted to Professor S.M. Alam, Head, Department of Zoology, Aligarh Muslim University, Aligarh, for encouragement and providing all possible facilities of work.

Special thanks are due to Dr. Jamil A. Khan for his unlimited courtesy; to Mr. Saleem Mustafa, for his cooperation in carrying out certain experiments and statistical analyses; and to all my friends and colleagues of the Section of Ichthyology and Fisheries, for their help whenever sought for. Financial assistance rendered by the Council of Scientific and Industrial Research, New Delhi, is also gratefully acknowledged.

GENERAL INTRODUCTION

Research on the biochemistry of fish is almost a century old. An impetus to investigations on the biochemistry and nutritive value of fish occurred after the World War I. Most studies were conducted on the vitamin A and D contents of fish tissue. The ravages of the World War destroyed the vast resources of protein food in many countries of the world, making the price of food beyond the reach of an average man. To meet this situation, dietitians and nutritionists initiated a search for a substitute food of high quality and yet cheap in price. Ultimately, fish was discovered as an ideal food. This stimulated early systematic investigations on fish.

The pioneer worker on the subject was Atwater (1888) who, along with his students at Wesleyan University in Connecticut, carried out thousands of chemical analyses from 1880 to 1890. The other notable contribution is that of Clark and Almy (1918) who analysed a large number of fishes from the Atlantic coast. These authors also investigated the seasonal variations in the proximate chemical composition of the fish. Another detailed study was carried out by Johnstone (1915, 1917, 1918a, b; 1920) on herring and other clupeoids.

Thereafter, many other workers carried out proximate chemical analyses of fish tissues (Bailey, 1942; Bailey et al. 1952; Dyer and

Fraser, 1963; Idler and Bitners, 1958, 1960; Mannan et al. 1961a,b).

Other important contributions in this field are those of Bergstrom (1961), Cartoni and Aloi (1934), El-Saby (1934), Kondo et al. (1941), Levanidov (1950), Love (1957), Luhmann (1953), Meyer (1958), Salques (1934), Stansby (1954), Thompson (1959), Tsuchiya et al. (1953) Valenzuela (1928).

In addition to these, a large number of analytical results have been compiled in the form of nutritional tables (Love et al. 1959; McCance and Widdowson, 1940; Taylor and MacLeod, 1949; Vinogradov, 1953).

Investigations on the chemical composition and nutritive value of Indian fishes have been initiated in recent years. The early work was carried out on fishes from the coastal regions of the country. The earliest detailed record on the chemical composition of Indian fishes are on Bengal fishes. (Basu and De, 1938; Saha and Guha, 1939, 1940). Niyogi et al. (1941) studied the chemical composition of 18 species from the Bombay coast. Appana and Devadatta (1942) analysed 4 specie of Bombay and Konkan coast. Setna et al. (1944) carried an investigation on fishes from the commercial catches of Bombay coast. Patakoot et al. (1950) analysed 32 species of common marine fishes from Bombay coast. Airan (1950) studied the protein and mineral composition of 7 species of Kolhapur freshwater.

Studies on similar lines have been carried out by Chari (1948)

on fishes of the Madras coast. Natrajan and Sreenivasan (1961) have studied the mineral composition of as many as 36 species of Bhavanisagar and Mettur reservoirs of Madras. Similar contributions in this field are from Chidambaram et al. (1952), Sekharan (1955), Sreenivasan and Natrajan (1961), Vasavan et al. (1960) and Venkataraman and Chari (1951, 1953).

Interesting studies have also appeared on the amino acid makeup of fish muscle (Ambe and Sohonie, 1957; Bose et al., 1959; Durairaj, 1961; Kulkarni, 1953; Master and Magar, 1954; Valanju and Sohonie, 1957a and b; Velankar and Govindan, 1957; Venkataraman and Chari, 1957).

A great deal of informations have been compiled on the biochemical composition and nutritive value of fishes from north India by Jafri et al. (1964) and Khawaja (1966).

At a very early stage, however, men must have learned the survival value of storage and seasonal surplus. Fish decay so rapidly that storage in itself presupposes some form of preservation. The sea fishes, whose bones are found in refuse heaps of Late Old Stone Age Cave dwellers in Dordogne, dating from about 40,000 B.C., must have been preserved in some way to have travelled so far inland. One can only guess that people were practising only drying of fishes in sun and wind, when the climate was suitable, or even in smokey fires. The direct evidence of the latter are found in wood, as deposites found alongside the River Bann in Northern Ireland, might have been the result of Salmon smoking. At last, we conclude that sun drying and smoke drying

were ancient practices.

After a long practice of consuming the salted, dried and smoked fishes, there appeared considerable interest in people of modern age to prefer fresh fish to salted, smoked or dried fish. Consequently, the history of development of fishing industry in modern times has been a succession of attempts to keep the fish fresh between catching and consumption. The improvement in the productivity of fishing lead to the need for improved methods of preservation. Since the catching stations are far away from the consuming places, people preferred to take fishes which were fresh or nearest in quality to the live fish. Since keeping fishes in live condition for considerable period was much more difficult, the process of preservation came into existence. Preservation was first met by the use of natural ice.

The freezing of the fish by natural means has long been known as a method of preservation in cold countries, like Russia, Siberia, Canada, Great Lake area and Newfoundland, where ice is easily available.

The earlier workers in the field of storage and preservation of fishes are fewer in number, but the practice is considerably old (Bate-smith, 1973; Birdseye, 1929; Dyer, 1951; Huntsman, 1929, 1931; Leim et al., 1927; Macpherson, 1932; Plank et al., 1916; Reuter, 1916; Seagran, 1956; Stevenson, 1899; Tressler and Evers, 1957; Zaitsev and Pavlov, 1958; Zaretschenzeff, 1930).

A great deal of work has been done on the factors affecting the

amino acid composition of fish (Jone, 1954). The important contributors in this field are Castell et al. (1970, 1974), Dyer et al. (1962, 1963, 1964, 1968), Fraser et al. (1961), Hiltz et al. (1973), Hodgkiss and Jones (1955), MacCallum (1964), MacCallum et al. (1964), Ranke (1950), Siebert (1958), Tarr (1968), Tomlinson et al. (1961, 1963, 1966), Tomlinson and Geiger (1962, 1963a, b).

Other important investigators in the field of spoilage and preservation are Charm et al. (1972), Connell and Howgate (1969), Ogata et al. (1972), Partmann (1963), Takama et al. (1972) and Warrier et al. (1972).

In India though fish is an important diet for a large proportion of population, information on changes in the biochemical composition and nutritive value of fish after they have been stored or preserved is meagre and in many cases rather incomplete. Although a few studies have been made on fishes and shell fishes of coastal regions (Fatema and Nagar, 1961; Jadhav and Nagar, 1970; Madhavan et al., 1970; Nazir and Nagar, 1965; Shinoy and Pillai, 1971; Valenkar, 1964; Vasantha et al., 1972; Venugopal et al., 1973), information on the freshwater fishes is much more limited (Annamalay, 1962; Menon, 1962).

The present study, which forms a part of this dissertation, includes the changes in the proximate chemical composition and free amino acids of an economically important freshwater murrel, Ophicephalus punctatus Bloch. during a short period of storage at room temperature (32 ± 2°C) and during freezing (-4 ± -4°C).

O. punctatus forms an extensive and persistent fishery which flourishes in all the seasons in the plains of Northern India. The fish is brought to the Aligarh fish market in large drums and earthenwares from catching stations. Although a major portion of the catch is sold in fresh condition, a substantial part is stored, either as such or under ice, for its subsequent transport to other markets. The storage of the fishes may singly or collectively impair the nutritive value of priced catch. Unfortunately, the consumer and the poor fisherman are completely unaware of all such changes. It is, therefore, hoped that the present study would be of interest to consumers, traders and dietitians.

PROCEDURE AND METHODOLOGY

Live Ophicephalus punctatus of the size range 17-21 cm were obtained from some local ponds of Aligarh and kept in aerated laboratory aquaria. At the time of investigation, the fishes were divided into two batches and killed by decapitation. One batch of the fish was stored at a temperature of $-4 \pm -4^{\circ}C$ and the other at $32 \pm 2^{\circ}C$. The total period of storage was 25 hours. After an interval of 5 hours each muscle sample was separately obtained from the trunk region of the three fishes. Care was taken to remove the bony elements from the muscle. It was then macerated in a high speed grinder and processed for various estimations.

METHODS OF ESTIMATION

MOISTURE

For the determination of moisture content, a known amount of muscle (5-10 g) was taken in weighed silica crucible and kept in an electrical oven at $100^{\circ}C$ for about 20 hours (A.O.A.C., 1960), till it became completely dry. The crucible was then taken out, cooled in a dessicator and weighed. The process was repeated till a constant weight was obtained. The loss of weight gave the index of moisture, from which the percentage of moisture was calculated.

ASH

The amount of ash was determined according to the standard technique given in the A.O.A.C. (1960). A weighed amount of sample (5-10 g) was taken in a silica crucible and dried in an oven at 100°C. It was then ignited at a temperature of 550°C, till the ash became white and completely free from carbon. The crucible, after cooling in a dessicator, was weighed and the percentage of ash in the sample calculated.

TOTAL FAT

Fat in fish tissue has been extracted by various investigators using different solvents. Solvent like ethanol-ether mixture is known to be effective in splitting fat from protein but dissolves certain non-fatty substances also and thus gives relatively high fat values. On the other hand, solvent such as petroleum ether which dissolves only the fat may give low values, since it is not so effective in splitting the fat protein linkage and possesses a poor wetting power (Jafri, 1965). Lovern (1955) has, therefore, suggested extraction of tissue first with a good wetting solvent like ethanol-ether mixture and then with petroleum ether. In the present study, fat was extracted with petroleum ether (B.P. 40-60°C). Although this method gives only an approximate estimate of the fat (crude fat) in tissue, yet it is effective wherever routine analysis on a large scale is attempted.

Muscle sample, completely dried in an oven, was mixed and ground thoroughly. Sample of known weight was teken in an extraction thimble, tightly plugged with cotton wool and extracted with petroleum

ether (B.P. 40-60°C) in soxhlet apparatus for about 10 hours. The solvent was distilled off from the oily extract by keeping the receiving flask on a water bath. The remaining traces of the solvent were finally removed in an oven. The flask was then cooled in a dessicator and weighed. The increase in the weight of the receiving flask gave the amount of fat extracted from the known quantity of sample. For ascertaining a complete extraction of the fat, the whole process was continued till no increase in the weight of the flask was obtained. A complete tissue extraction took nearly 10 hours.

PROTEIN

Protein was estimated by a slight modification of Wong's (1923) micro-kjeldahl method. Muscle tissue, weighing 0.1 g, was digested in 5 ml of nitrogen free 1:1 sulphuric acid, using potassium persulphate as an oxidizing agent. The digestion in sulphuric acid results in the conversion of all the nitrogenous materials into ammonium sulphate. The digested sample was diluted to 50 ml with distilled water. A known aliquot of this solution was then directly nesslerized. The mercuric iodide contained in Nessler's reagent combines with the ammonium sulphate of the digested solution, producing a brown coloured solution of oxydimercuric iodide, $(OHg)_2 NH_2I$. The intensity of the colour developed was proportional to the amount of ammonium sulphate solution. A blank sample was also prepared in the same way for comparing the colour intensity on a Bausch and Lomb Spectronic 20 Spectrophotometer at 480 mu wave length.

A calibration curve of ammonium sulphate was prepared by taking readings of a series of solutions which always contained a known amount of nitrogen. The readings obtained from the unknown solution were read against the ammonium sulphate calibration curve. The amount of nitrogen obtained was multiplied by the protein factor (6.25) to obtain the value of the protein content.

QUALITATIVE ANALYSIS OF FREE AMINO ACIDS

The free amino acids were extracted after the methods of Krishnamoorthy (1958) and Block et al. (1958). The muscle sample (5 g) was taken in a glass mortar and homogenized in 85% alcohol. The insoluble material was removed by centrifugation at 3000 rpm for about 15 minutes and washed with 85% alcohol. Three volume of chloroform was then added to each volume of ethanol extract. After thorough shaking, the mixture was again centrifuged at 3000 rpm for 15 minutes and the resulting aqueous (upper) layer was taken out with a syringe and dried in an oven at 65 $\pm$ 5° C for 24 hours. The dried material was then dissolved in 10% isopropanol and used for final spotting.

A known quantity (3 ul) of the amino acid solution extracted from the muscle, and a same amount of 10 mM solution of the known amino acids in 10% isopropanol (Block et al., 1958), were applied by micro-pippete as compact spots on Whatman filter paper sheet (28.5 x 46 cm). The method of ascending-one-dimentional chromatography was followed. A mixture of n-butanol-glacial acetic acid-water (4:1:5) was used as solvent. The chomatogram was run for nearly 18 hours. The paper was

then removed from the chromatographic chamber and dried in a strong current of air, sprayed with 0.25% ninhydrin in acetone (W/V) and dried at 65 $\pm$ 5^{o}C in an electric oven for 15 minutes in order to get the maximum colour development. The spots of individual amino acids were identified by comparing their Rf values with those of known amino acids developed on a separate chromatogram under identical conditions.

CHAPTER I

CHANGES IN THE PROXIMATE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF THE FRESHWATER MURREL, *OPHICEPHALUS PUNCTATUS* BLOCH. DURING STORAGE

PART I : PROTEIN

INTRODUCTION

From a nutritive point of view, protein is the most essential component of diet. Fish is one of the best sources of animal protein, containing almost all the essential amino acids necessary for growth and metabolism. The relative amount of muscular tissue is also generally higher in fish than in domestic animal or man (Hamoir, 1955; Jacquot and Creach, 1950).

Studies on the nutritive value of fish protein were initiated in many countries of the world immediately after the World War I, when an acute shortage of protein food occurred.

Systematic investigation on the nutritive value of the fish protein were initiated in 1918 by Drummond. Later, Suzuki *et al.* (1919) carried out interesting investigations on the protein of sardine. Kik and MacCollum (1927) investigated the quality of haddock and herring protein. Evaluation have been made of the proteins of some Indian fish species by Airan and Joshi (1952).

Fig. 1 Changes in the muscle protein content during storage at $-4^{0}C$.

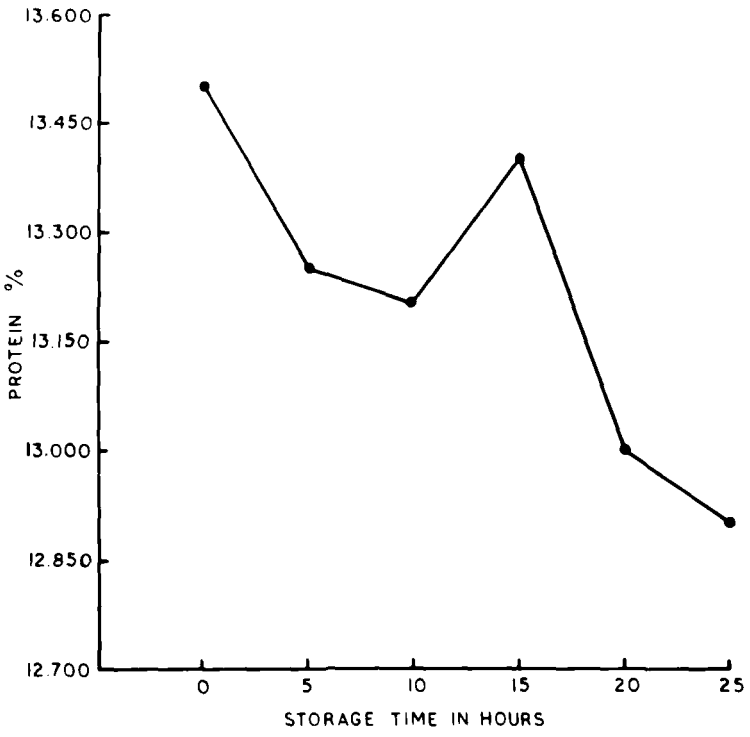
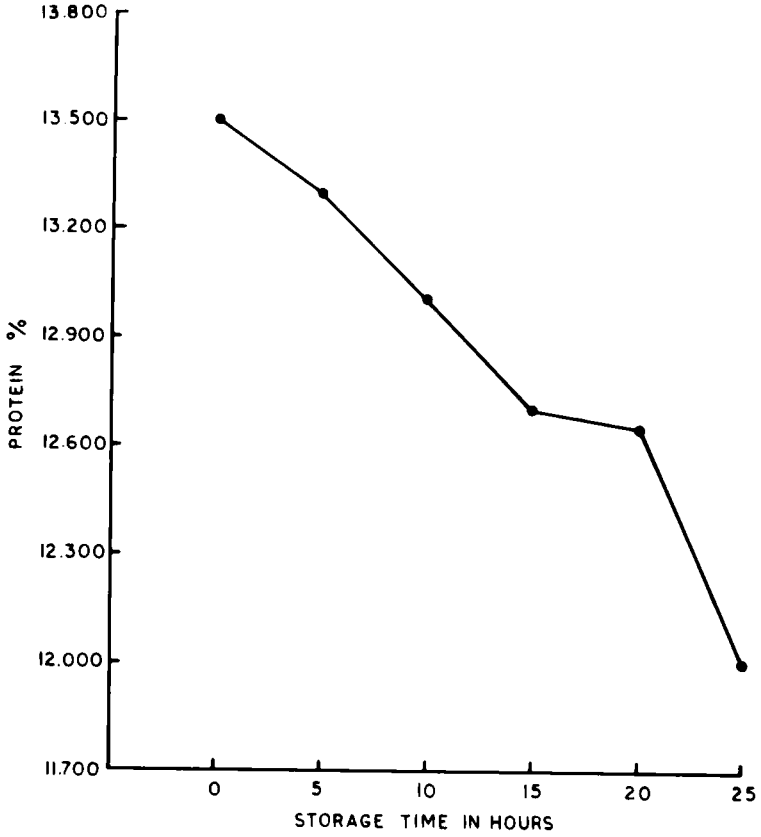

13.600
13.450
13.300
13.150
13.000
12.850
12.700
PROTEIN %
0
5
10
15
20
25
STORAGE TIME IN HOURS

Fig. 2 Changes in the muscle protein content during storage at 32°C.


13.800
13.500
13.200
12.900
12.600
12.300
12.000
11.700
PROTEIN %
0
5
10
15
20
25
STORAGE TIME IN HOURS

Other studies concerning the biochemical characteristics of the fish muscle protein are those of Weber and Mayer (1933) and Roth (1947). Investigations on the changes in the nutritive value of fish protein during storage and freezing were carried out by several workers in the past(Dyer, 1964; Fraser, 1961; Reay et al., 1943; Shewan and Jones, 1957; Stewart, 1935). Similar investigations on Indian fishes were relatively few (Jadhav and Nagar, 1970; Madhavan et al., 1970; Shiney and Pillai, 1971; Vasantha et al., 1972).

The present chapter deals with the changes in the protein content of the freshwater murrel, Ophicephalus punctatus Bloch., during different storage conditions.

RESULTS AND DISCUSSION

The protein values during the successive periods of storage, from 5 hours to 25 hours, have been statistically evaluated, presented in Tables I and II and shown graphically in Figs. 1 and 2.

It can be seen from the data that the freshly stored fish possessed a muscle protein value of 13.500% but declined gradually to 12.00% after 25 hours of storage at 32°C (Table II; Fig. 2). The fishes kept at -4°C, however, showed a decline from 13.500% to 12.900% during a 25 hour storage (Table I). Thus, the loss in the muscle protein value, though not very marked, was found to increase with the lapse of time and was comparatively more in fishes stored at 32°C than in those kept at -4°C (Fig. 1, Table I).

The decline in the calorific value, related to protein, of

Q. punctatus during a 25 hours storage at 32°C was found to be from 58.05 to 51.600 calories/100 g of tissue, incurring a net loss of about 7 calories. At -4°C the decline in calorific value for the same period of storage was recorded to be from 58.05 to 55.470 calories/100 g of tissue. The net loss incurred in this condition was about 4 calories.

The loss in protein content recorded during the present investigation may be attributed mainly to factors like the decomposition of amino acids and exudation of free drip (Tarr, 1942). Indeed, when the fishes are improperly preserved, the microbial decomposition may affect the amino acid content of the flesh protein (Borgstrom, 1962).

The taste, flavour and the nutritive value of stored fish depends upon the amino acid make-up of the protein molecules, since each species of fish has an 'amino acid pool' in the intracellular system of the muscle which is specific and does not show obvious changes even under varying environmental conditions (Florkin, 1949, 1955). A minor change may, however, take place in the pool of the amino acids under the influence of varying temperature. Since certain amino acids govern the taste of fish as pointed out earlier, the development of bad odor and flavour in the fish seems to be indicative of the biochemical changes in those amino acids. At low temperature, however, no marked changes in amino acid composition has been reported in fish muscle. Ingalls et al., (1950) found no appreciable difference in the essential amino acid composition of frozen meat. Freezing also did not affect the

nutritive value of crab meat as determined biologically (Watson and Feller, 1935).

Drip has also been reported to be an important phenomenon causing a considerable loss in the muscle protein of fish during storage. The loss of protein through drip has been found to be greater in frozen than in unfrozen fish (Moore et al., 1970). More or less similar results were obtained during the present studies on O.punctatus (Tables I and II; Figs. 1 and 2). The protein loss through drip has also been recorded by Seagran (1958).

PART II : AMINO ACIDS

INTRODUCTION

Nutritional studies on fish protein have largely been confined to the proteins of the muscle and their amino acids. The typical taste of the fish depends upon its characteristic amino acid pool. The amount of amino acid is, to a large extent, responsible for the degree of spoilage in fish. Fishes having a higher quality of amino acids are reported to spoil more rapidly (Banks, 1959).

There are varied literature on the post-mortem changes in the amino acid make-up of fishes. The effect of various storage conditions on the free amino acid composition of fishes have been studied by Hodgkiss and Jones (1955) and Jones (1954). Amano and Hito (1950) have shown the sequence of free amino acids generated from the decomposition of fish. A considerable amount of literature has accumulated on the study of histamine. Histamine has been used as an index of the freshness of fish (Borgstrom, 1961). Studies have also been made on the changes in the general chemical composition of the fish protein during different storage conditions. The effect of storage condition on denaturation of protein has been worked out by Finn (1932, 1934) and Reay (1933, 1934, 1935). Love (1956) has shown the effect of the rate of freezing on the

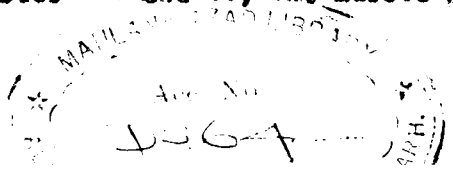
denaturation of fish protein. Seagran (1956) studied the chemical changes in fish actomyosin during freezing and storage. Asakawa (1957), Reay (1949), Saito and Hidaka (1957) and Yoshimura et al. (1953) have investigated the changes in protein molecules after death. Dingle et al. (1955) have made interesting eletrophoretic studies of the proteins extractable from post-rigor cod at low ionic strength solution.

A good deal of work on the subject has also been done on the marine fishes of India. Velankar and Govindan (1958) found free nitrogen content as an index of iced prawn. Noorjani et al., (1957) assessed the quality of fishes by different methods. Basu and De (1938) analysed the hydrolysed fish protein from wastes of fishes. However, information on the freshwater fishes is almost lacking.

The present chapter deals with the changes observed in the distribution of free amino acids in the muscle of the freshwater murrel, Ophicephalus punctatus Bloch., during a 25 hours storage at -4°C and 32°C.

RESULTS AND DISCUSSION

In the present study on the free amino acid pattern in the skeletal muscle of freshwater murrel, O. punctatus Bloch., some interesting changes have been observed during storage at 32°C and -4°C. On storing fishes at 32°C, bad or unpleasant odor was produced after 5 hours and this went on increasing during the later period of storage. As would be evident from Tables III and IV, the muscle protein of freshly preserved



O. punctatus showed the presence of leucine, isoleucine-phenylalanine, tryptophan, valine, methionine, alanine, glutamic acid-threonine, arginine-serine-aspartic acid, histidine, lysine, glycine and cystine. Tyrosine and proline were not seen in the fresh condition of the fish but suddenly appeared on storage. Leucine, isoleucine alanine, tyrosine and glycine were observed through all the stages of storage but there is a great deal of fluctuations in their relative concentrations. The pattern of changes in the distribution of free amino acid of O. punctatus stored at 32°C was more or less similar to those kept at -4°C.

As has already been pointed out, each species of fish possesses a specific "amino acid pool" which does not alter, even in varied conditions of environment (Florkin, 1949, 1955). The findings on free amino acid pattern of the murrel, O. punctatus Bloch., during different periods of storage, presented above, clearly indicate that, despite some similarities there is no definite trend in the pattern of changes of these free amino acids. It was observed that there was comparatively few drastic changes in the free amino acid pattern during 25 hours of storage at the two temperatures. Also, there was very little difference between the free amino acid pattern of fishes kept at -4°C and 32°C. Therefore, no particular inference with regard to the pattern of gradation of the free amino acid can be drawn. The presence of essential amino acids, such as leucine, phenylalanine, isoleucine, tryptophan, valine methionine, threonine, arginine and tyrosine, in the muscle of the fresh fish, as also throughout the period of storage, at the two temperatures, indicates

that there is no alarming loss in the nutritional value of the flesh of O. punctatus upto about 25 hours after death. The disappearance of certain amino acids at particular period of storage, however, may be due to deamination and reamination, respectively. The disappearance of certain amino acids, such as proline in O. punctatus stored at 32°C, at particular hours of storage may be due to their incorporation into other substances, creating bad odor or unpleasant flavour (Braastedt, 1957). The bad odor recorded during the present investigation in fishes kept at 32°C is perhaps an indicative to this fact. It can be seen that proline, though absent in freshly preserved O. punctatus, made its appearance at 5 and 10 hours of storage but disappeared at later stages. Since only bacteria present in the muscle can be causative to the deamination or transamination of free amino acids, they may also cause an increase or decrease in the amount of particular amino acid during storage.

Fig. 3 Changes in the muscle fat content during storage at $-4^{0}C$

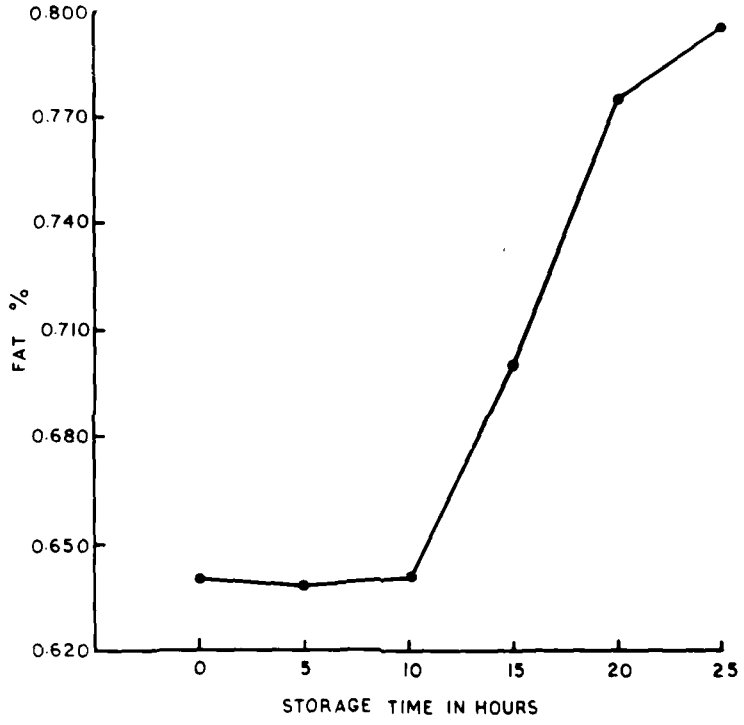
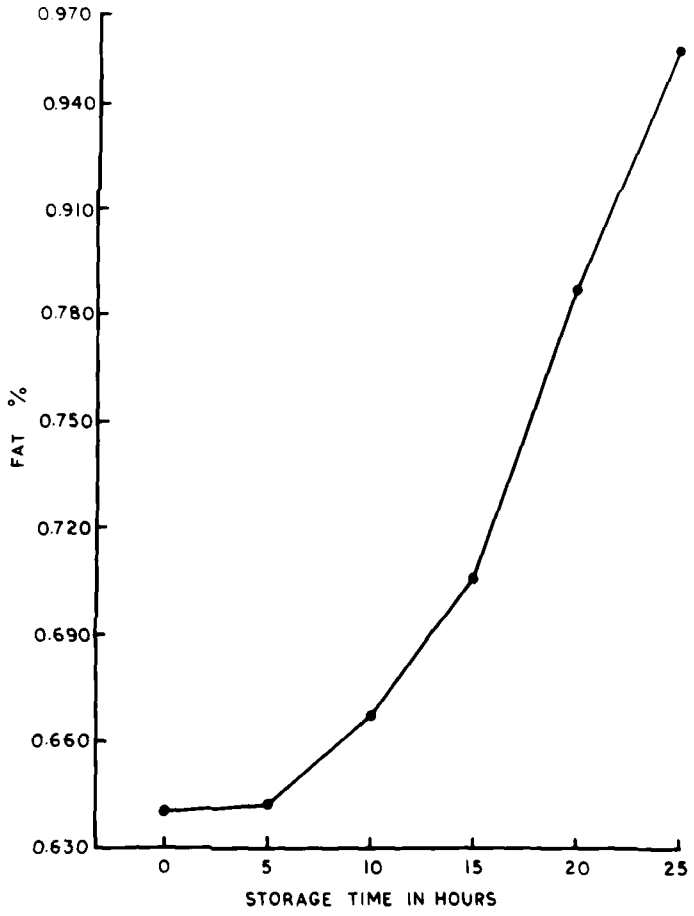

0.800
0.770
0.740
0.710
0.680
0.650
0.620
FAT %
0
5
10
15
20
25
STORAGE TIME IN HOURS

Fig. 4 Changes in the muscle fat content during storage at 32°C.


0.970
0.940
0.910
0.780
0.750
0.720
0.690
0.660
0.630
FAT %
0
5
10
15
20
25
STORAGE TIME IN HOURS

PART VII : FAT

INTRODUCTION

Information on changes in the physico-chemical quality of triglycerides of fish during various technological processes seems limited, presumably due to the complex nature of these triglycerides. A classical study on the nutritional significance of fish lipid was made by Lovern (1958). Some studies on the changes in fish fat during oxidation and on the development of rancidity have also appeared in the past (Bligh, 1961; Brown et al., 1956; Farmer et al., 1943; Gunstone and Hilditch, 1946; Maclean et al., 1964). However, any satisfactory report of this nature on the fishes, especially from the freshwater environment of India, is yet to be placed on record.

During the course of present investigations, some considerations have been made of the changes which occur in the fat content of muscle of the freshwater murrel, Ophiocephalus punctatus Bloch., during storage at different temperatures. The factors which singly or collectively impair the quality of fish fat have also been briefly discussed.

RESULTS AND DISCUSSION

The values of muscle fat content during different periods of storage at -4°C and 32°C have been given in Tables V and VI and plotted in Figs. 3 and 4.

The muscle of the freshly preserved O. punctatus was found to possess about 0.6% of fat. A marked rise in the fat content was observed with the lapse of time in the two batches of fishes kept at the two temperatures. It is interesting to note that this rise in the fat content was more rapid in fishes stored at a higher temperature (32°C) than in those kept at a low temperature (-4°C). A peculiar unpleasant smell also developed at early stages in fishes stored at the former temperature. No such smell could be observed in fishes kept at -4°C. The quantitative variations in muscle fat and the development of unpleasant odor observed during the present study, in fishes stored for various lengths at the two temperatures, may be attributed mainly to the oxidative changes occurring in the muscle. The differences in the degree of lipid oxidation under the two sets of conditions may be related to factors such as temperature, light and access to atmospheric oxygen.

The unpleasant odor and taste which develops in fish fat upon storage or aging is reported to be the result of oxidation (Bergstrom, 1961). The atmospheric oxygen is believed to attack the bond of the fatty acid to form a peroxide linkage. The fishy flavour may also be associated with the traces of peroxide, formaldehyde and tertiary nitrogen in the form of volatile bases, trimethylamine, trimethylamine oxide or a mixture of both (Brooe, 1941; Davies and Gills, 1936; Obata et al., 1949). Farmer and Sutton (1943) have ascribed the development of unpleasant fishy smell in the autoxidation of fatty acids and their esters to the breakdown products of highly oxidized unsaturated acids. Toyama and Matsumoto (1953) have reported the presence of volatile

substances of highly unsaturated fatty acids and carbonyl compounds but maintained that these may not be regarded as the chief substances responsible for the unpleasant odor peculiar to oxidized highly unsaturated fatty acids.

During the present observations on O. punctatus it was observed that the rise in the muscle fat content was more in fishes stored at 32°C than those stored at -4°C (Figs. 3 and 4). The percentage of rise was, however, considerably low during the initial stages of autoxidation, where perhaps peroxide began to be formed, than during the later stages, where peroxide may be decomposed, reacting with one another or with other oxidative products. Such reaction ultimately results in the formation of various acids, carbonyl compounds and other products (Borgstrom, 1961). The development of unpleasant flavour detectable during later stages of storage of O. punctatus at 32°C seems indicative of such reactions. In any case, the rise encountered in the muscle fat content of this fish seemed due to a marked accumulation of oxidative products with different physical properties and chemical constitution.

Among the factors influencing the rate of autoxidation, temperature seems to be of prime importance as became evident from the differences arising in the time of onset of unpleasant smell in O. punctatus stored at the two different temperatures. Light is also known to enhance the rate of oxidation and hence the peroxide portion.

Besides oxidation, the development of unpleasant odor in fish flesh upon storage may also be the result of hydrolysis of triglycerides,

resulting in the formation of glycerol and free fatty acids. The free fatty acids may account for the increase in the weight of the fat, such as has been observed during the present investigation in O. punctatus. The increase in the concentration of free fatty acids, as a part of post-mortem changes in fish, has been reported by a number of workers (Banks, 1937; Love, 1966; Lunde, 1939). In contrast to oxidative changes, lipid hydrolysis in fish by itself has no obvious nutritional significance. The accumulation of free fatty acids in fish tissue as such does not seem to affect the culinary quality of fish but affects are likely to be due to the secondary changes, particularly increased susceptibility of fat to oxidation and development of bad flavour (Lovern, 1962). The hydrolysis in fish tissue may be caused by factors such as enzymatic activities, bacterial activities, free fatty acid concentrations, etc. The hydrolytic activity may be enhanced at high temperatures but checked to some extent at low temperatures. In the worst stages, the additive effects of oxidation, enzymatic and bacterial hydrolysis of fats in fish may result in extremely bad odor and off-flavour responsible for reducing the consumer appeal of the fish (Ranke et al., 1957). At such stage, a loss of vitamins from tissue may also be encountered. It may, however, be mentioned that at a narrow range of temperature changes, storage of fish for a short period may not cause any marked changes in its nutritive value.

PART IV

WATER AND ICE

INTRODUCTION

Early studies on the freezing of water content of the tissue during storage at low temperatures were summerized by Birdseye (1929). Finn (1934) determined the amount of ice frozen out directly from the muscle pressed-juice of the halibut by separating the ice formed at successive low temperatures through filtration. Most of the older theories of tissue freezing describe a mechanical damage to the cell wall and structure by the formation of ice crystals of various sizes. Investigations on the ice formation in fishes during freezing conditions are well documented (Callow, 1952; Motevaro and Meen, 1940; Zarotschenzeff, 1930). Mackay and Weld (1926) and Weld (1927) showed that the average size of ice crystals formed during slow freezing were appreciably larger than those formed during rapid freezing. Fast freezing also results in the formation of larger number of ice crystals of small sizes, leaving the cells undamaged. Dieuzeide and Novella (1950), Mahadevan and Carter (1940) and Novella (1950) worked on the ice formation in haddock and concluded that nearly 80% of the water freezes.

Fig. 5 Changes in the muscle water content during storage at -4^{0}C.

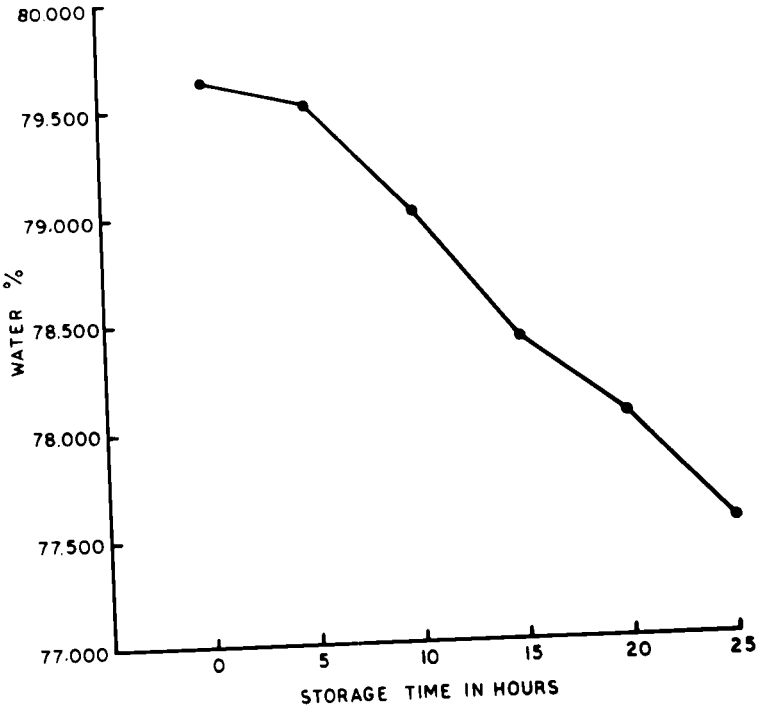
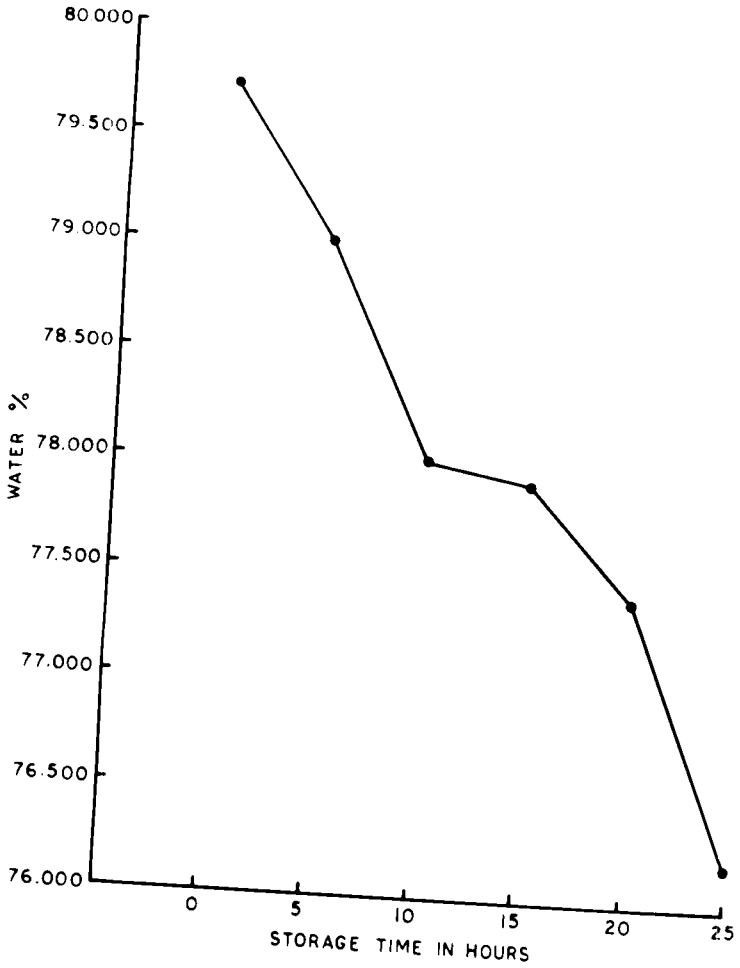

80.000
79.500
79.000
78.500
78.000
77.500
77.000
WATER %
0
5
10
15
20
25
STORAGE TIME IN HOURS

Fig. 6 Changes in the muscle water content during storage at 32°C.


80.000
79.500
79.000
78.500
78.000
77.500
77.000
76.500
76.000
WATER %
0
5
10
15
20
25
STORAGE TIME IN HOURS

In addition to this free water, a little amount of water is bound with different organic and inorganic materials. Dyer (1951) noticed a dessication of water from fish surface due to improper wrapping of the fish. The effect of concentration of salts present in the cells of fish was thoroughly studied by Duerr and Dyer (1952). Love (1955a, 1956a, b and c) has attempted to measure the drip in fishes by estimating the intracellular deoxyribose nucleic acid.

The percentage of ash in fish muscle have been estimated in the past by many workers (Forastrom, 1961). The amount of ash present in some Indian freshwater fishes have been reported by Jafri et al. (1964). Although ash forms an important component of fish tissue, nothing has appeared, in detail, on the technological significance of this constituent in fish processing.

The present work describes the changes in the water and ash contents of Ophicephalus punctatus Bloch, during storage at -4°C and at 32°C for 25 hours.

RESULTS AND DISCUSSION

WATER

It is evident from Tables VII and VIII and Figs. 5 and 6 that the percentage of water in the muscle of O. punctatus showed a decrease in fishes stored at the two temperatures (-4°C and 32°C). In freshly stored fish, the muscle was found to contain 79.720 % water. This declined to 76.200 % in fishes stored at 32°C, and to 77.620 % in fishes

stored at -4°C during a period of 25 hours (Figs. 5 and 6). The rate of decrease of water in fishes placed at -4°C was, however, little lower than in those placed at 22°C.

The loss in the amount of water is the main cause of dehydration from the surface due to improper wrapping of fishes. Consequently, dehydration of protein and complete denaturation of salt-soluble protein in the surface layer of fish occurs (Dyer, 1951). Decrease in the moisture has also been suggested by Finn (1929) who explained that due to a freezing out of water, pH was lowered which would be expected to decrease the net charge of the proteins and render the hydrophobic. The increasing salt concentration during freezing results in the splitting up of actomyosin into actin and myosin, as evidenced by the fact that extracts from the stored fish contained no actomyosin (Borgstrom, 1961). At low temperatures, ionic concentration should reach salting out range of proteins present. However, there will be an increased viscosity and a reduced molecular diffusion, resulting in lower rate of aggregation than those at high temperatures. Also, protein gel will be more concentrated and proximity of protein may allow the London Van Der Waals' forces to come into play, but higher temperatures result in more aggregation of protein.

The drip formed in the storage tenure of fish appears to contain water in addition to salts and other constituents. The formation of drip is intracellular as evidenced by the observation of Love (1958a, 1958a, b and c) who measured the cell damages by estimating the TMA

Fig. 7 Changes in the muscle ash contents during storage at $-4^{0}C$.

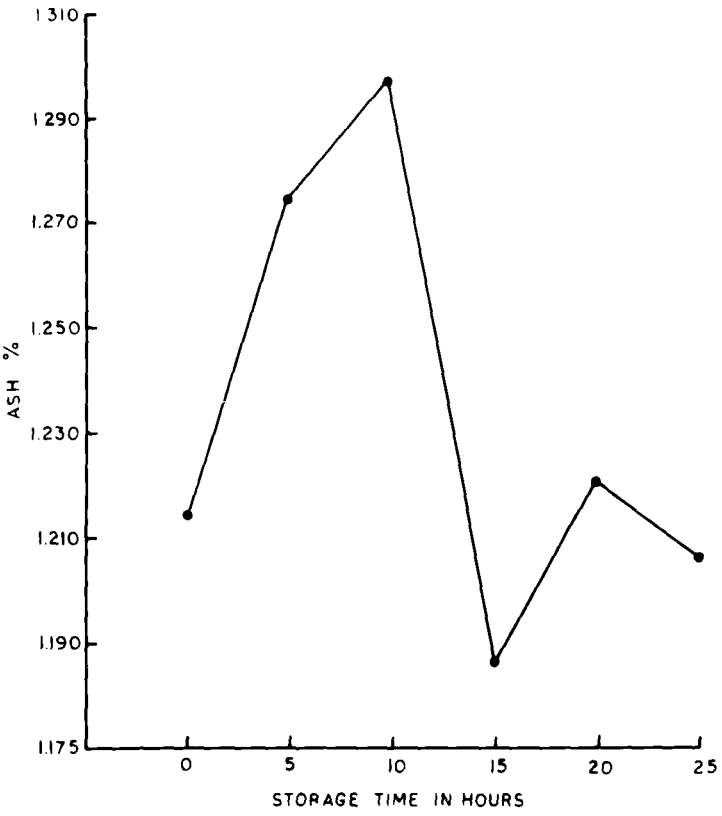
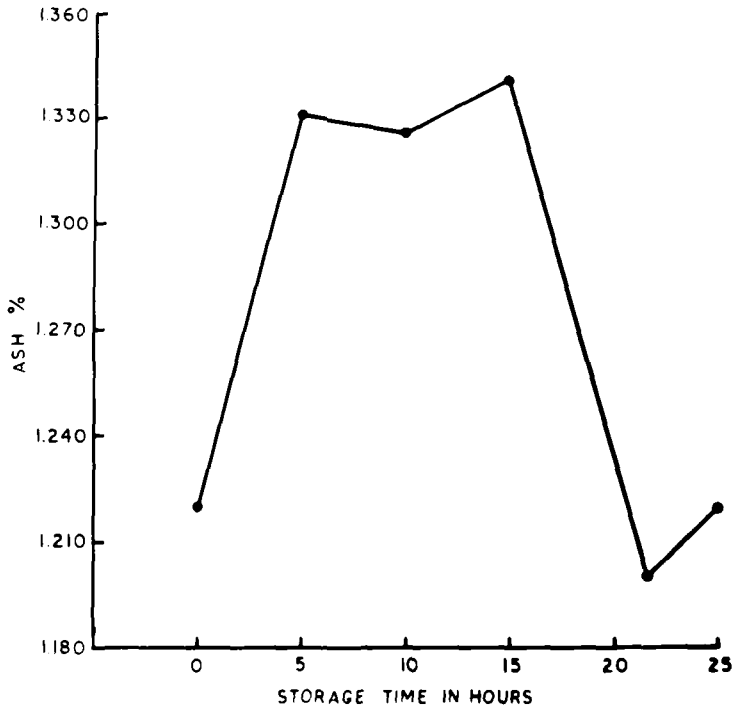

1.310
1.290
1.270
1.250
1.230
1.210
1.190
1.175
ASH %
0
5
10
15
20
25
STORAGE TIME IN HOURS

Fig. 8 Changes in the muscle ash content during storage at 32°C.


1.360
1.330
1.300
1.270
1.240
1.210
1.180
ASH %
0
5
10
15
20
25
STORAGE TIME IN HOURS

released from the bursting of cells.

Other factors may also be involved in causing a gradual decrease of water from fish tissue during storage. It, however, seems difficult to single out the various factors as due to the freezing out of water, salt concentration increases and many of the inorganic ions present in the drip combine with lactic acid, formed as a result of post-mortem glycolysis, and form various compounds such as lithium lactate, calcium lactate, etc. Besides these compounds, the post-mortem deterioration of fish is also characterized by the formation of trimethylamine formed by the reduction of trimethylamine oxide. TMO is hygroscopic and its loss through the drip is variably accompanied by the loss of some tissue water (Borgstrom, 1961).

ASH

The values of ash in fishes stored for various durations at different temperatures have been given in Tables IX and X and plotted in Figs. 7 and 8.

The trend of changes recorded for the ash content differed considerably from these observed for other constituents. The percentage of ash in fishes stored at -4°C and 32°C showed an initial rise up to a certain period after which a sharp decline in the ash content was evident (Figs. 7 and 8). The variations in the ash content may be the result of degree of dehydration of fish tissue encountered during storage. It has been reported in the past that the various processing

operations do not result in the loss of mineral elements (Borgstrom, 1962). However, if there is any loss, it may be entirely physical, due to liquid discarded from fish products(MacLance and Shipp, 1933).

GENERAL SUMMARY

Ophicephalus punctatus Bloch., a common freshwater murrel, showed interesting changes in its proximate chemical composition, amino acid make-up and nutritive value during storage for a short period (25 hours) at different temperatures (-4°C and 32°C). The freshly stored fish was found to be rich in protein, but a gradual decline in the protein percentage occurred with the lapse of time at the two temperatures. This decline was, however, more pronounced in the fishes stored at a higher temperature. A marked fall in the calorific value of the protein content was also observed. Some interesting changes were observed in the pattern of the distribution and relative concentrations of free amino acids of the fish during the storage. The variations in the amino acids were, however, more or less similar at the two temperatures. Muscle fat content, on the other hand, registered a marked rise in the two batches of the fishes, being more rapid in fishes stored at higher temperature. The distribution of water seemed to follow a general inverse relationship with that of fat during the 25 hours storage. The percentage of ash in the muscle showed an initial rise, after which a sharp decline was evident. An unpleasant odor also developed in fishes during early stages of storage at the higher temperature. The present observations indicate that the fish during initial period of its storage,

at low temperature, was rich in all its important biochemical constituents, including amino acids, and that no undesirable change occurred in its calorific value. The significance of the observed changes was briefly discussed.

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TABLE - I

Changes in the muscle protein percentage of O.punctatus during storage at -4° C.

Storage time in hrs.	Mean Protein%	S.D.	C.V.	S.E.M.	Variance
0	13.500	0.418	3.0962	0.2413	0.1747
5	13.250	0.1113	0.8400	0.0642	0.0123
10	13.200	0.1178	0.8920	0.0671	0.0138
15	13.400	0.0710	0.5299	0.0405	0.0050
20	13.000	0.1452	1.1169	0.0838	0.0210
25	12.900	0.1417	1.0984	0.0808	0.0200

S.D. = Standard Deviation
C.V. = Coefficient of variation
S.E.M. = Mean Standard Error

TABLE - II

Changes in the muscle protein percentage of *O.punctatus* during storage at 32^{o} C.

Storage time in hrs.	Mean Protein %	S.D.	C.V.	S.E.M.	Variance
0	13.500	0.4180	3.0962	0.2413	0.1747
5	13.300	0.0866	0.6511	0.0500	0.0074
10	13.000	0.3500	2.6923	0.2020	0.1225
15	12.700	0.1228	0.9669	0.0709	0.0150
20	12.650	0.0908	0.7177	0.0534	0.0082
25	12.000	0.1802	1.5016	0.1040	0.0324

TABLE - III

Pattern of free amino acid distribution in the muscle of *O. punctatus* during storage at -4°C

AMINO ACIDS	0/hour	5/hours	10/hours	15/hours	20/hours	25/hours
Leucine	++	+++	+	++	++	+++
Isoleucine-Phenylalanine	+	++	+	++	++	++
Tryptophan	++	-	-	-	-	-
Valine-Methionine	+	+	-	++	+	+
Tyrosine	-	+	-	+	+	++
Proline	-	++	-	+	+	++
Alanine	+++	++	+	+	++	+
Glutamic acid-Threonine	+	+	+	++	++	+++
Arginine-Serine-Aspartic acid	++	+++	-	++	+	+++
Histidine	+++	++	-	++	+	++
Lysine	+	++	+	+	+	++
Glycine	+	+	++	+	+	++
Cystine	+	+++	-	-	+	++

TABLE - IV

Pattern of free amino acid distribution in the muscle of *O. punctatus* during storage at 32°C

AMINO ACIDS	0/hour	5/hours	10/hours	15/hours	20/hours	25/hours
Leucine	++	+++	++	++	+++	++
Isoleucine-Phenylalanine	+	+	++	++	++	++
Tryptophan	++	-	-	-	-	-
Valine-Methionine	+	+	+	+++	++	++
Tyrosine	-	+	+	-	++	++
Proline	-	-	-	+	++	+++
Alanine	+++	++	++	++	+++	+++
Glutamic acid-Threonine	+	+	+	+	++	+++
Arginine-Serine-Aspartic Acid	++	+++	++	++	-	-
Histidine	+++	++	++	-	++	+++
Lysine	+	++	++	++	+++	+++
Glycine	+	+	+	++	++	++
Cystine	+	++	-	-	-	-

TABLE - V

Changes in the muscle fat percentage of O. punctatus during Storage at -4°C

Storage time in hrs.	Mean Fat%	S.D.	C.V.	S.E.M.	Variance
0	0.640	0.0150	2.3437	0.0086	0.00225
5	0.638	0.0111	1.7398	0.0064	0.0001?
10	0.640	0.0050	0.7812	0.0028	0.0000?
15	0.700	0.0091	1.3000	0.005?	0.00008
20	0.773	0.0061	0.7891	0.0035	0.00003
25	0.794	0.0086	1.0831	0.0049	0.00007

TABLE - VI

Changes in the muscle fat percentage of O. punctatus during storage at 32° C.

Storage time in hrs.	Mean Fat%	S.D.	C.V.	S.E.M.	Variance
0	0.640	0.0150	2.3437	0.0086	0.00225
5	0.642	0.0148	2.3052	0.0085	0.00219
10	0.667	0.0072	1.0794	0.0041	0.00005
15	0.706	0.0091	1.2889	0.0052	0.00008
20	0.788	0.0046	0.5837	0.0026	0.00002
25	0.856	0.0210	2.4647	0.0121	0.00044

TABLE - VII

Changes in the muscle water percentage of O. punctatus during Storage at -4° C.

Storage Time in hrs.	Mean water %	S.D.	C.V.	S.E.M.	Variance
0	79.720	0.2025	0.2540	0.1169	0.04100
5	79.500	0.2156	0.2711	0.1244	0.04648
10	79.000	0.3122	0.3951	0.1802	0.09746
15	78.460	0.1819	0.2320	0.1050	0.03308
20	78.120	0.0648	0.8294	0.0374	0.00419
25	77.628	0.3420	0.4405	0.1970	0.11696

TABLE - VIII

Changes in the muscle water percentage of O.punctatus during storage at 32° C.

Storage time in hrs.	Mean Water %	S.D.	C.V.	S.E.M.	Variance
0	79.720	0.2025	0.2540	0.1169	0.04100
5	79.000	0.0705	0.0892	0.0407	0.00497
10	78.000	0.0975	0.1251	0.0562	0.00950
15	77.980	0.1113	0.1427	0.0642	0.01238
20	77.460	0.0781	0.1008	0.0450	0.00609
25	76.200	0.0872	0.1144	0.0503	0.00760

TABLE - IX

Changes in the muscle ash percentage of *O. punctatus* during storage at -4° C.

Storage time in hours	Mean Ash %	S.D.	C.V.	S.E.M.	Variance
0	1.215	0.0055	4.5267	0.00288	0.00002
5	1.273	0.1247	9.7957	0.0719	0.01555
10	1.299	0.1663	12.8021	0.0960	0.02765
15	1.187	0.1934	16.2931	0.1116	0.03740
20	1.220	0.1131	9.2704	0.6653	0.01279
25	1.207	0.1284	10.6379	0.0741	0.01648

TABLE - X

Changes in the muscle ash percentage of *O.punctatus* during storage at 32°C.

Storage time in hrs.	Mean Ash%	S.D.	C.V.	S.E.M.	Variance
0	1.215	0.0055	4.5267	0.0028	0.00002
5	1.331	0.0110	0.8264	0.0063	0.00012
10	1.326	0.0066	0.4977	0.0038	0.00435
15	1.342	0.1001	7.4590	0.0577	1.00200
20	1.200	0.1060	8.8330	0.0612	1.12360
25	1.220	0.1116	9.1475	0.0644	1.24545